Program/Abstract # 54
The Misshapen kinase negatively regulates integrin levels to promote collective cell migration in Drosophila
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In the Drosophila ovary, each egg chamber elongates along its A–P axis to transform these initially spherical structures into highly elongated eggs. During this process, the follicle cell (FC) epithelium displays an unusual planar polarity at its basal surface that is independent of the Frizzled-PCP pathway. This polarization coincides with a newly described motility in this tissue, in which the FCs undergo a directed migration that causes the entire egg chamber to rotate around its A–P axis as it lengthens (Haigo and Bilder, 2011). Both FC planar polarity and motility appear to be required for elongation morphogenesis, as mutations that disrupt these processes produce spherical eggs; however, little is known about the mechanisms underlying this phenomenon. Through a forward genetic screen, we have identified the Misshapen (Msn) kinase as a key regulator of egg chamber elongation. Interestingly, there is a position-specific effect to the loss of Msn within the FCs. Msn mutant clones in medial egg chamber regions disrupt the planar arrangement of basal actin filaments and ECM molecules. In contrast, mutant clones at the egg chamber termini have no effect on FC planar polarity, and instead lead to a novel phenotype, in which wild-type cells at the clone border detach from their mutant neighbors and invade the germ cells. These disparate phenotypes can be explained by our finding that mutant FCs show increased integrin levels at their basal surface and adhere more tightly to the ECM. Our data suggest that Msn promotes FC planar polarity and migration by negatively regulating integrins, and highlighting the importance of precisely regulating adhesion levels across an epithelium during collective cell behaviors.

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Program/Abstract # 55
Phosphoinositide(3,5) bis phosphate is essential for formin-mediated polarized growth
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Tip growth is a highly polarized form of cell expansion found in all plants. Although restricted to a few cell types, tip growth is essential for the development of plant species ranging from algae to flowering plants. In the moss *P. patens* the colonizing tissue emerges from the spore and propagates by tip growth. The apical cell is tip growing and is the stem cell for this stage of development. We are using moss to study tip growth due to the ease of molecular genetic manipulation and the abundance of tip growing cells. The moss system is also unique for its gene-targeting capabilities as well as rapid RNAi, which can reveal plant gene function in one week. Using RNAi coupled with complementation studies, we have shown that class II formins, cellular nucleators and elongators of actin filaments, are essential for tip growth. Class II formins are composed of an N-terminal phosphatase tensin (PTEN) and a C-terminal FH1–FH2 domain. The PTEN domain is related to PTEN lipid phosphatases, and mediates formin localization near the apex of the cell and at the site of cell division. The FH1–FH2 domain is required for actin nucleation/elongation. Here, we show that the PTEN domain binds PI(3,5)P2 and that this interaction is essential for formin function. Using chimeric proteins, where the N-terminal PTEN domain was replaced with polypeptides of known lipid binding specificity, we show that PI(3,5)P2 is necessary and sufficient for formin-mediated tip growth. However unlike the PTEN domain, the polypeptides that functionally replaced PTEN are not enriched at the cell apex or cell division site. This result therefore unlinked formin localization from function. Instead, using variable angle epifluorescence microscopy (VAEM), we discovered that class II formins localize, via their PTEN domain, to dynamic cortical dots. Importantly, functional PI(3,5)P2 binders share this localization, suggesting that class II formin function requires interaction with PI(3,5)P2 containing membranes at the cell cortex.

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Program/Abstract # 56
Planar polarity signaling negatively regulates neurite formation to maintain neuronal morphology in *C. elegans*
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The first overt sign of polarization in newly born neurons is neurite emergence followed by differentiation of these neurites into axons and dendrites. While molecules that orient nascent neurite growth along specific trajectories are beginning to be elucidated, the cellular and molecular pathways that prevent inappropriate neurite formation in order to maintain polarized neuronal morphology and prevent extraneous connections are poorly understood. To gain further insight into this process, we performed an unbiased forward genetic screen for specific determinants of neuritogenesis and not general growth cone motility or axon guidance factors. The VC neurons are a set of six peripheral motor neurons with bipolar morphology and stereotypical differences in the orientation of neurite growth along the anterior–posterior (A–P) body axis: VC1-3 and VC6 project neurites along the A–P axis, whereas VC4 and VC5 project neurites along the orthogonal left-right (L–R) axis generated by the developing vulva, an intermediate guidepost tissue during innervation of egg-laying organ targets. Our analysis has revealed a previously unknown inhibitory role for a planar cell polarity (PCP) pathway consisting of the *C. elegans* orthologs of Van Gogh (VANG-1), Prickle (PRKL-1), and Dishevelled (DSH-1) in maintaining the polarity of nascent neurite emergence along the normal axis of extension by blocking extraneous neurite formation along an orthogonal axis. In vang-1, PRKL-1, and dsh-1 mutants, VC4 and VC5 neurons display aberrant 'tripolar' morphologies due to inappropriate neurite growth along the A–P body axis in addition to normal neurite extension along the L–R vulval axis. This loss of normal neuronal morphology increases gradually with developmental time suggesting a primary disruption in polarity maintenance rather than establishment. We found that autonomous and non-autonomous PCP signaling is required early and persistently to inhibit the formation or consolidation of leading edge protrusions directed away from vulval guidepost cells. Several findings point to a key role for PRKL-1 in this process. PRKL-1 hyperactivation in VC4 and VC5 restores normal polarity in vang-1 and dsh-1 mutants. Furthermore, PRKL-1 overexpression but not that of VANG-1 or DSH-1 is sufficient to suppress neurite formation in VC4 and VC5 and reorient the A–P polarity of VC6 in a vang-1 and dsh-1-dependent manner. These findings suggest a novel role for PCP signaling in maintaining polarized neuronal morphology by blocking neuronal responses to neuritogenic cues that would otherwise promote extraneous neurite formation.

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